CASEIN KINASE- 1 EPSILON DELETION ENHANCES OPIOID REWARD AND IS ASSOCIATED WITH INCREASED STRIATAL OPRM1 AND NPAS4 EXPRESSION

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ABSTRACT

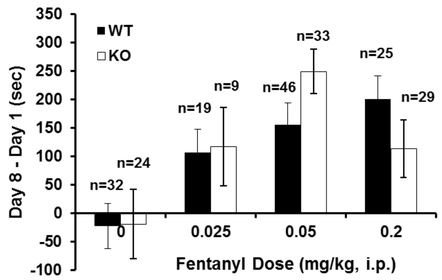
Genetic and pharmacological studies indicate that Casein kinase-1 epsilon (*Csnk1e*) contributes to psychostimulant, opioid, and ethanol behaviors. Pharmacological inhibition of CSNK1E enhanced psychomotor sensitivity to the selective mu-opioid receptor agonist fentanyl, indicating a negative regulatory role in drug behavior. Here, we tested the hypothesis that *Csnk1e* negatively regulates fentanyl reward using the conditioned place preference assay (CPP) in *Csnk1e* knockout (KO) and wild-type (WT) mice. KOs showed a leftward shift in the inverted u-shaped curve for opioid reward versus WTs, exhibiting enhanced reward at lower doses (0.05 mg/kg) and decreased reward at higher doses (0.2 mg/kg). No significant differences were observed in fentanyl analgesia in the 52.5°C hot plate assay (0-0.4 mg/kg) or naloxone conditioned place aversion (4 mg/kg), suggesting a neural mechanism selective for dopaminergic reward circuitry. To generate novel hypotheses regarding the molecular mechanisms that mediate enhanced opioid reward in *Csnk1e* KOs, we used transcriptome analysis via mRNA sequencing of striatum from naïve KO and WT mice to identify the transcription factor *Npas4* as the top hit (2.2-fold increase in expression; p=4.96 x10^{-136}), supporting *Npas4* transcript covariance with morphine reward (Piechota et al., 2010). Importantly, expression of *Oprm1* (mu-opioid receptor) was higher in KOs compared to WTs (1.7-fold increase; p=2.14x10^{-4}). The activity of one of the top upstream regulators identified by Ingenuity Pathway Analysis, STAT3, is negatively regulated by CSNK1E and *Stat3* has previously been shown to regulate *Oprm1* expression. We conclude that *Csnk1e* deletion enhances opioid reward, possibly via a STAT3-mediated increase in mu-opioid receptor expression.

RESULTS

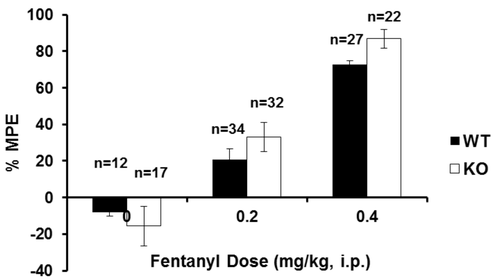
Csnk1e KOs showed enhanced fentanyl reward compared to WTs at a low dose (0.05 mg/kg) and a reduction in fentanyl reward at the highest dose (0.2 mg/kg), suggesting an enhanced sensitivity to the aversive properties of opiates that are observed following high doses. No differences were observed in fentanyl analgesia in the 52.5°C hot plate assay (0-0.4 mg/kg) or naloxone conditioned place aversion (4 mg/kg). Combined, these results suggests that Csnk1e regulates drug-induced behaviors via a dopaminergic mechanism. The mRNA sequencing experiment yielded 408 differentially expressed genes (DEGs), including *Npas4* as our top hit and *Oprm1*. Ingenuity Pathway Analysis (IPA) identified Behavioral, Development Disorders and Endocrine Disorders as the top gene network. Additionally, IPA identified STAT3 as one of the top upstream regulators. Geneweaver analyses identified 5 genes (Rnf7, Trim71, Aldh1a2, Oprm1, and Ifi203) from our DEG list that overlapped with publicly available gene sets that correlated with drug-induced behaviors. The results from our side-by-side signaling and behavior experiment, do not provide evidence for altered fentanyl-induced phosphorylation of DARPP-32 in Csnk1e knockout mice compared to wild-type mice.

1. Enhanced sensitivity to opioid reward/aversion in Csnk1e knockout mice.

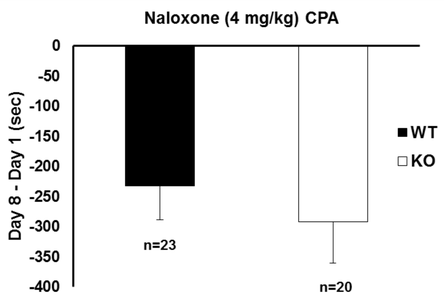
Day1	Day2 Day3	Day4 Day5	Day6 Day7	Day8
SAL	DRUG SAL	DRUG SAL	- -	SAL
Initial pref.	Training		Consolid.	CPP



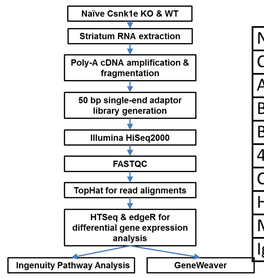
2. Opioid analgesia in Csnk1e knockout mice.



3. Csnk1e knockout mice do not display differential opioid antagonist conditioned place aversion

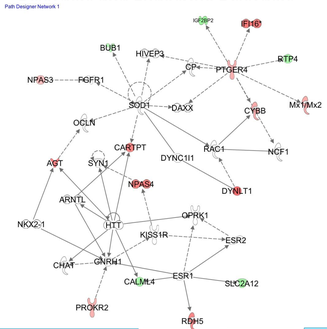


4. Striatal transcriptome analysis and subsequent bioinformatic analyses in Csnk1e knockout mice.

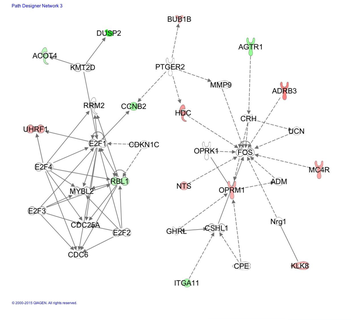


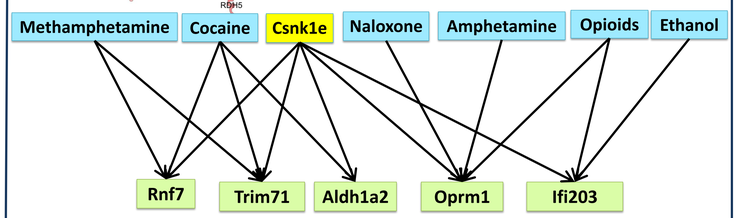
	FC	PValue	FDR
Npas4	2.246	3.38E-140	4.96E-136
C030023E24R	-0.641	2.74E-68	2.01E-64
Agt	2.268	4.02E-27	1.47E-24
Btg2	1.697	1.28E-24	3.53E-22
Baiap3	1.649	8.55E-24	1.96E-21
4931408D14R	-0.184	4.89E-21	7.63E-19
Ccno	1.967	7.08E-21	1.07E-18
Hist2h3c1	-0.551	2.28E-20	3.21E-18
Mvp	1.683	1.85E-17	1.76E-15
Igsf1	2.662	2.73E-17	2.53E-15

Network 1: Behavioral, Developmental Disorders and Endocrine Disorders



Network 3: Behavior, Cell Cycle, and Digestive Development and Disorders

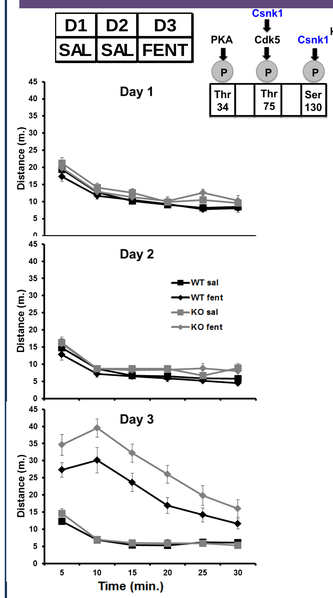


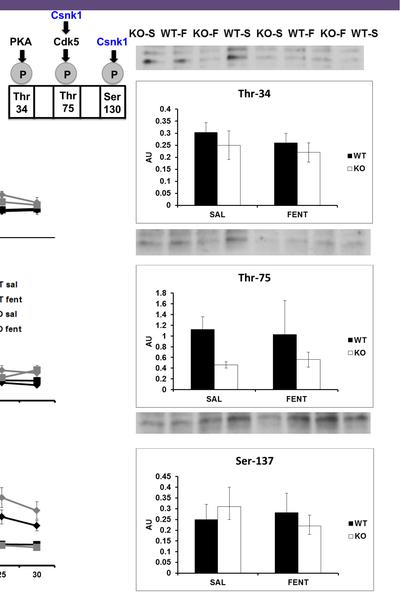


MATERIALS AND METHODS

Csnk1e KO and WT mice were bred by heterozygote breeding. Twenty-four hours post-assessment of initial preference for the drug-paired side on Day 1, mice received fentanyl (CPP: 0, 0.025, 0.05, 0.2 mg/kg, i.p.) or naloxone (CPA: 4 mg/kg) on Days 2 and 4 on the drug-paired side, and saline (i.p.) on Days 3 and 5 on the other side (distinguished by floor textures). Seventy-two hours later (Day 8), mice were assessed for either fentanyl CPP or naloxone CPA (Day 8-Day 1). For the hot plate assay, mice were assessed for pain response (licking the hindpaw) on the hot plate (52.5°C) prior to drug exposure, and 10 minutes after fentanyl injection (0, 0.2, 0.4 mg/kg, i.p.). Striatum from naïve KO and WT was utilized for mRNA sequencing. A 50 bp single-end adaptor library was generated and run on an Illumina HiSeq 2000. FastQC was completed and Tophat was utilized for read alignment. DEGs were identified using HTSeq and EdgeR with sex as a covariate. For the locomotor activity and signaling experiment, mice received saline (i.p.) on Days 1 and 2, and either saline (i.p.) or fentanyl (0.2 mg/kg, i.p.) on D3. Striatum was collected immediately following D3 and samples will be flash-frozen and processed for western blotting. Immunoblotting for total DARPP-32 (1:1000), Thr34-DARPP-32 (1:1000), Thr75-DARPP-32 (1:1000), and Ser130-DARPP-32 (1:1000) was completed.

5. Differential fentanyl-induced locomotor activity and DARPP-32 phosphorylation in Csnk1e knockout mice





SUMMARY

- Csnk1e KOs showed enhanced fentanyl CPP at a low dose (0.05 mg/kg,i.p.) and decreased CPP at the highest dose (0.2 mg/kg,i.p.) compared to WT suggesting enhanced sensitivity to both the rewarding and aversive properties of opiates
- No differences were observed in fentanyl analgesia in the 52.5°C hot plate assay (0-0.4 mg/kg, i.p.) or naloxone conditioned place aversion (4 mg/kg, i.p.).
- Fentanyl induced increased locomotor activity in KOs compared to WTs but no concomitant differences in DARPP-32 phosphorylation were observed
- Csnk1e KOs show increased *Npas4* and *Oprm1* expression
- IPA identified STAT3 as a top upstream regulator
- The activity of STAT3 is negatively regulated by CSNK1E and STAT3 positively regulates the expression of *Oprm1*
- Geneweaver identified 5 genes that overlap between our DEG list and drug-induced gene sets, suggesting a role of *Csnk1e* in regulating the behavioral effects of multiple classes of drugs of abuse
- We conclude that Csnk1e deletion enhances opioid reward, possibly via a STAT3- or *Npas4*-mediated increase in mu-opioid receptor expression.

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